proteins (Figure 1A). Considering the significant differences between the methods used, and the analysis of distinct hESC lines between studies, the 35% overlap detected seems to be of some significance, and in line with previous comparisons (Olsen et al., 2006). At the same time, given the different methods of differentiation utilized by the two groups, the fact that BMP4 induction promotes extraembryonic lineages (Xu et al., 2002; Pera et al., 2004) whereas retinoic acid directs hESCs toward an ectoderm cell fate (Wichterle et al., 2002), and differing time points examined in each case, it should not be surprising that we see much less overlap (7%) between the two differentiated data sets (Figure 1B). However, it is interesting to note that a significant number of protein synthesis and translation regulators are found differentially phosphorylated within only 240 min of differentiation (a majority within as few as 30 min) (Figure 1B). This pattern implies that FGF removal and/or addition of BMP4 also regulate the cell at the translational level in addition to the transcriptional level.

Needless to say, both papers provide a substantial amount of data that stem cell scientists can mine for the purpose of developing new hypotheses. These models can then be tested for their potential roles in the control of the undifferentiated state or in the initial steps of differentiation. Brill et al. (2009) undertake this approach by identifying additional receptor tyrosine kinases activated in hESCs and, in doing so, reveal an effect of PDGF in the maintenance of pluripotency. Meanwhile, Van Hoof et al. (2009) identify a phosphorylation site on Sox2 that mediates SUMOylation, potentially providing a mechanism to overcome the stem cell regulatory circuitry during the initial phase of differentiation.

Many of the phosphorylation sites identified in these studies remain uncharacterized, and their functions unknown, and yet describing these data sets is only an initial step in characterizing the hESC phosphoproteome, given that Swaney et al. (2009) have recently identified thousands more sites. Ultimately these types of studies will provide sufficient phosphoproteome resources to allow the stem cell community to integrate cellular regulation at all levels of control and achieve mastery over the hESC and its fate choices. It may be a daunting task, but it is exciting to see the progress made thus far.

REFERENCES

Brill, L.M., Xiong, W., Lee, K.B., Ficarro, S.B., Crain, A., Xu, Y., Terskikh, A., Snyder, E.Y., and Ding, S. (2009). Cell Stem Cell *5*, this issue, 204–213.

Olsen, J.V., Blagoev, B., Gnad, F., Macek, B., Kumar, C., Mortensen, P., and Mann, M. (2006). Cell *127*, 635–648.

Pawson, T., and Nash, P. (2003). Science *300*, 445–452.

Pera, M.F., Andrade, J., Houssami, S., Reubinoff, B., Trounson, A., Stanley, E.G., Ward-van Oostwaard, D., and Mummery, C. (2004). J. Cell Sci. *117*, 1269–1280.

Sampath, P., Pritchard, D.K., Pabon, L., Reinecke, H., Schwartz, S.M., Morris, D.R., and Murry, C.E. (2008). Cell Stem Cell *2*, 448–460.

Swaney, D.L., Graeme, C., McAlister, G.C., Joshua, J., and Coon, J.J. (2008). Nat. Methods *5*, 959–964.

Swaney, D.L., Wenger, C.D., Thomson, J.A., and Coon, J.J. (2009). Proc. Natl. Acad. Sci. USA *106*, 995–1000.

Van Hoof, D., Munoz, J., Braam, S.R., Pinkse, M.W.H., Linding, R., Heck, A.J.R., Mummery, C.L., and Krijgsveld, J. (2009). Cell Stem Cell *5*, this issue, 214–226.

Wichterle, H., Lieberam, I., Porter, J.A., and Jessell, T.M. (2002). Cell *110*, 385–397.

Xu, R., Chen, X., Li, D.S., Li, R., Addicks, G.C., Glennon, C., Zwaka, T.P., and Thomson, J.A. (2002). Nat. Biotechnol. *20*, 1261–1264.

CD95/Fas in the Brain—Not Just a Killer

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Although CD95 (Fas/Apo-1) has long been known to be broadly expressed in the brain, its function has remained enigmatic. In this issue of *Cell Stem Cell*, Corsini et al. (2009) now show that CD95 serves as a potent activator of neurogenesis in both the healthy and injured brain.

CD95 is the best characterized and paradigmatic member of the TNF-receptor superfamily of "death receptors," and the molecular mechanism of CD95-induced apoptosis is known specifically. After binding of CD95L, CD95 forms trimers and sequentially recruits the adaptor protein FADD, regulatory proteins (like DAXX or FLIP), and procaspase 8, leading to the formation of a death-inducing complex (DISC). The oligomerization then results in the autoproteolytic cleavage of procaspase 8 and the initiation of the apoptotic cascade (Peter and Krammer, 2003). In the central nervous system, CD95 expression varies significantly during development. In the adult brain, neurons in the hippocampus and cerebral cortex show the highest CD95 expression, although CD95 expression is also detectable on astrocytes and oligodendrocytes especially under pathological conditions. Conversely, the cognate ligand CD95L is constitutively coexpressed on neurons

and perivascular astrocytes of the healthy brain and upregulated by ischemia and inflammation. Mice with defective CD95 receptor signaling perform better and develop smaller lesions compared with their wild-type littermates in several models of acute brain damage including stroke, multiple sclerosis, and trauma, and such a result is consistent with CD95 receptor signaling's role in apoptosis (Reich et al., 2008).

The well-established proapoptotic effect of CD95 raises the intriguing question why CD95 is also constitutively expressed by neurons in the healthy brain. A lifelong expression just to kill neurons once a stroke happens appears rather unlikely. In addition, a detailed analysis of CD95-deficient mice showed significantly impaired spatial learning of healthy and injured animals, suggesting more complex roles for CD95 signaling in the adult brain (Reich et al., 2008; Sakic et al., 1997). In two recent papers, Ana Martin-Villalba's group has now investigated the physiological function of CD95 in the brain apart from inducing apoptosis. Their studies reveal that CD95 can potently mediate neuroregeneration by both inducing dendrite branching of mature neurons (Zuliani et al., 2006) and by activating adult neurogenesis (Corsini et al., 2009).

Corsini et al. (2009) report that CD95 promotes neurogenesis and favors neuronal differentiation at the neural stem cell (NSC) level but does not induce apoptosis in this issue of *Cell Stem Cell*. On a functional level, experimental suppression of CD95-mediated neurogenesis in the hippocampus resulted in reduced spatial learning of untreated animals and impaired integration of injected NSCs into the hippocampus in a mouse model of global ischemia. Interestingly, the data presented suggests that CD95 expression is restricted on the stem cell fraction within the subventricular zone (SVZ). This high specificity may even allow the use of CD95 as NSC-specific marker and implies a distinct proneurogenic function of CD95L/CD95 interactions within the stem cell niche. A comparative analysis will help to integrate the CD95/CD95L system with other molecular signaling cascades such as EGF or BMP that are also known to regulate neurogenesis in the SVZ.

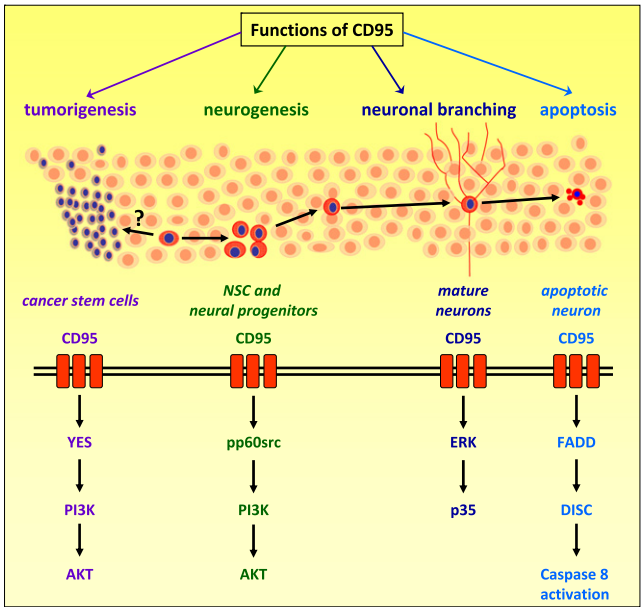

Figure 1. Differential Function of CD95 during Neurogenesis
The figure shows the known and postulated functions of CD95 during neurogenesis and gives an overview over the respective intracellular signaling pathways identified so far.

In the present work, Corsini et al. (2009) have identified some of the components of the intracellular signaling cascade necessary for CD95-mediated neurogenesis (see Figure 1). In NSCs, activation of CD95 by CD95L induced the recruitment of the scr-familiy kinase member pp60-src and the activation of the PI-3 kinase/AKT signaling pathway. Notably, this signaling pathway was found to occur in the absence of formation of the apoptotic DISC and also did not require the recruitment of the adaptor protein FADD. The promotion of neurogenesis further required the phosphorylation and the subsequent activation of two additional downstream proteins: GSK3β and mTOR. The intracellular signaling via PI-3 kinase/AKT differs from a previously described CD95-meditated neuronal branching pathway that requires the activation of the ERK-p35 signaling pathway but was also found to be independent of the recruitment of FADD and the activation of caspases (Desbarats et al., 2003).

The spectrum of different CD95-activated apoptotic and nonapoptotic intracellular signaling cascades in different neural populations and different stages of neurogenesis demonstrates the biological importance of a tight regulation of CD95 during each neuron's life. CD95-associated regulatory proteins like PEA-1, Rac1, c-FLIP, and FAF1 and the neuron-specific group of FAIM proteins including FAIM1 (short/long) and FAIM2 (LFG/NMP35) play a major but only partially characterized role in regulating the responsiveness of NSC, neuroblasts, and neurons toward CD95L-mediated apoptotic and nonapoptotic signaling (Beier et al., 2005; Reich et al., 2008). It will be exciting to understand how these proteins guide CD95 to induce cell death, neurogenesis, or neurite outgrowth. The work by Corsini et al. (2009) now provides the starting point for additional in-depth investigation of this intracellular network at all stages of neurogenesis.

The work from Martin-Villalbas group on the context-dependent functions of CD95 also has possible implications for the genesis of glioblastoma cancer stem cells (CSCs, also referred to as glioblastoma-initiating cells) that probably derive from NSC (Sanai et al., 2005). In contrast to its proapoptotic function in postmitotic neurons, CD95-mediated nonapoptotic signaling in NSCs may even bear oncogenic potential (Corsini et al., 2009). Mutations in the CD95 signaling pathway resulting in deregulated proliferation of NSC because of an increased proportion of asymmetrical cell divisions of NSCs and a decreased neuronal differentiation after activation of CD95 could potentially result in (pre-) cancerous lesions and may thus be involved in the transition of NSCs into glioblastoma CSCs. In this case, the function of CD95 may not switch from "neurogenic" toward "apoptotic" but toward "oncogenic." In line with this idea, the promigratory function of CD95 on glioma cells was only recently discovered by the same group (Kleber et al., 2008).

After having analyzed the "dark sides" of CD95 in brain and in glioblastoma (Kleber et al., 2008; Martin-Villalba et al., 1999), the group of Ana Martin-Villalba has now provided evidence that CD95 is not just a killer but can also be beneficial for brain repair (Corsini et al., 2009; Zuliani et al., 2006). Thus, targeting CD95-mediated neurogenesis could potentially be a useful strategy for the treatment of a wide range of neurological diseases. However, the three-faceted role of CD95 in the brain also makes this receptor a dangerous therapeutic target. Taken together, the recent body of work on the complex role of CD95 in the brain suggests that, the intracellular regulatory network of CD95 may be instrumental in regulating whether CD95 will act to stimulate proapoptotic, proneurogenic, or even protumorigenic signaling pathways. Additional work to further elucidate the regulatory network of CD95 will thus be needed to identify specific targets downstream of CD95 that will serve to direct the CD95 inducing signal specifically along neuroregenerative pathways while bypassing apoptosis.

REFERENCES

Beier, C.P., Wischhusen, J., Gleichmann, M., Gerhardt, E., Pekanovic, A., Krueger, A., Taylor, V., Suter, U., Krammer, P.H., Endres, M., et al. (2005). J. Neurosci. *25*, 6765–6774.

Corsini, N.S., Sancho-Martinez, I., Laudenklos, S., Glagow, D., Kumar, S., Letellier, E., Koch, P., Teodoreczyk, M., Kleber, S., Klussmann, S., et al. (2009). Cell Stem Cell *5*, this issue, 178–190.

Desbarats, J., Birge, R.B., Mimouni-Rongy, M., Weinstein, D.E., Palerme, J.S., and Newell, M.K. (2003). Nat. Cell Biol. *5*, 118–125.

Kleber, S., Sancho-Martinez, I., Wiestler, B., Beisel, A., Gieffers, C., Hill, O., Thiemann, M., Mueller, W., Sykora, J., Kuhn, A., et al. (2008). Cancer Cell *13*, 235–248.

Martin-Villalba, A., Herr, I., Jeremias, I., Hahne, M., Brandt, R., Vogel, J., Schenkel, J., Herdegen, T., and Debatin, K.M. (1999). J. Neurosci. *19*, 3809–3817.

Peter, M.E., and Krammer, P.H. (2003). Cell Death Differ. *10*, 26–35.

Reich, A., Spering, C., and Schulz, J.B. (2008). Trends Neurosci. *31*, 478–486.

Sakic, B., Szechtman, H., and Denburg, J.A. (1997). Neurosci. Biobehav. Rev. *21*, 327–340.

Sanai, N., Alvarez-Buylla, A., and Berger, M.S. (2005). N. Engl. J. Med. *353*, 811–822.

Zuliani, C., Kleber, S., Klussmann, S., Wenger, T., Kenzelmann, M., Schreglmann, N., Martinez, A., del Rio, J.A., Soriano, E., Vodrazka, P., et al. (2006). Cell Death Differ. *13*, 31–40.

Smooth(ing) Muscle Differentiation by MicroRNAs

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In a recent report in *Nature*, Cordes et al. (2009) demonstrate that miR-143 and miR-145 modulate smooth muscle cell (SMC) plasticity in part by regulating key transcription factors involved in SMC fate determination.

Recent studies have established microRNAs (miRNAs) as a class of critical mediators involved in the regulation of cell proliferation and differentiation in cardiac (van Rooij et al., 2007; Zhao et al., 2007), skeletal (Chen et al., 2006), and smooth (Cheng et al., 2009) muscles. In addition, miRNAs have also been demonstrated to play a role in the maintenance of embryonic stem cell (ESC) pluripotency (Xu et al., 2009). Cordes et al. (2009) now link the function of miRNAs to smooth muscle cell (SMC) fate determination and plasticity by showing that miR-143 and miR-145 regulate the proliferation and differentiation of vascular SMCs.

During the early embryonic stages of vasculogenesis, SMCs and their progenitors are highly proliferative and migratory. However, in adult blood vessels, SMCs become quiescent and express a repertoire of contractile, calcium regulatory, and signal transduction proteins necessary for the contractile function of fully differentiated SMCs (Owens et al., 2004). Further, SMCs, unlike cardiac and skeletal myocytes, are not terminally differentiated and are capable of regaining their highly proliferative and migratory characteristics under certain conditions such as vascular injury. Expression of nearly all SMC marker genes is known to be dependent upon one or more serum response elements (SRE, $CC(AT)_6GG$ or CArG boxes) in their promoters/enhancers. Serum response factor (SRF) is known to regulate growth response genes as well as muscle-specific genes through its interaction with the muscle cell-enriched SRF cofactor myocardin (Wang et al., 2001). Further, it is well documented that SRF cofactors, many of which are antagonistic in action, are mechanistically involved in regulating phenotypic switching of SMCs between proliferation and differentiation, thus providing a molecular explanation of cell fate maintenance and change at the transcriptional level (Owens et al., 2004; Wang et al., 2004). However, the functional significance of miRNAs during SMC differentiation remains uncertain. In particular, whether a specific miRNA is both necessary and sufficient to induce